

Telomerase is the Key to Maintaining the Length of Telomeres

Telomerase Regulation

Telomerase activity is very low in mammalian cells and absent in most types of terminally differentiated somatic cells (cells that won't change even during proliferation). High telomerase activity exists in germ cells, stem cells, epidermal skin cells, follicular hair cells, and cancer cells. (Ishikawa 1997) Some studies have suggested telomerase-activity regulation at each stage of the cell cycle. Dionne used a strain of yeast, lacking telomerase activity, to address the role telomerase plays in the formation of telomere replication intermediates *in vivo*. He mutated the gene encoding the RNA portion of telomerase. Extracts prepared from yeast carrying this mutation lacked telomerase activity. Researchers then analyzed the telomerase-negative yeast for the presence of telomeres. Telomeres formed during the S phase of the cell cycle (DNA replication phase), without telomerase. These results suggest the formation of telomeres is cell cycle dependent and telomeres can form without telomerase. (Dionne 1996) Experimental results reported by Zhu also support cell cycle regulation of telomerase activity. His research team arrested human tumor cell lines at different stages of the cell cycle. Cells arrested at the G1 phase of the cell cycle had similar telomerase levels as non-arrested cells at the same stage. Progression through the S phase of the cell cycle brought telomerase levels to their highest point, and levels declined in the G2 phase. (Zhu 1996) Other researchers have reported results that do not support the hypothesis that telomerase activity is cell cycle dependent.

In 1996, Shawn Holt reported the down-regulation of telomerase activity in telomerase-competent cells during quiescent periods (G phases of the cell cycle), and up-regulation at the onset of proliferation (mitosis). In 1997, Holt reported results in which he examined immortal cultured cell lines in an attempt to resolve these discrepancies. He found that telomerase activity did not change significantly with progression through the stages of the cell cycle. Telomerase activity declined in cells whose growth rate declined and was almost absent in cells that exited the cell cycle. Telomerase activity correlated with growth rate and repression activity in quiescent cells. (Holt 1997) Telomere degradation, through incomplete DNA synthesis, poses no threat to cells that are not dividing, and cells in a quiescent or differentiated state may have other means of regulating telomerase activity.

Results published by Cassandra Belair support Holt's belief that telomerase activity correlates with growth rate. Belair measured telomerase activity in two cell types. Isogenic (having an identical set of genes), normal human uroepithelial cells (HUCs), and cells from biopsies of superficial (on the surface) and myoinvasive (extending into the muscle) transitional cell carcinoma (TCC) cancer of the bladder. Non-cultured and cultured cells of each type provided information related to proliferative (multiplying) and nonproliferative cells. All carcinoma cells demonstrated telomerase activity. Uncultured, non-proliferating HUCs were telomerase negative and cultured HUCs, exhibiting a normal proliferation rate, were telomerase positive at lower levels than the TCCs. These results demonstrate accelerated proliferation of the TCCs. (Belair 1997) Another researcher, Weng, has found evidence of telomerase activity induction by various cellular signals.


Weng and his coworkers reported telomere length and telomerase activity in human B cells during differentiation. B lymphocytes derive from bone marrow progenitor (ancestor) cells and undergo an ordered differentiation with distinct stages during development and activation of in immune response. During T cell dependent immune responses, mature B lymphocytes enter a unique environment called the germinal center (GC). These cells differentiate into GC B cells and

then into memory B cells. The introduction of an antigen and Interleukin 4 initiated the immune response. B cells within the GC had significantly longer telomeres and the telomerase activity was 128 times higher than the naive (undifferentiated) and memory B cells (differentiated). They reported these cells capable of up-regulating telomerase activity, *in vitro*, in response to CD40 antibody/antigen receptor binding and Interleukin 4 (part of the cells machinery for fighting infection). (Weng 1997) The ability to chemically induce telomerase to lengthen telomeres could have profound effects on aging research and treatment. Another possible mechanism of telomerase regulation appeared last year in the Proceedings of the National Academy of Science.

Bhattacharyya and Blackburn reported results of their examination of telomerase non-template RNA domains. When they added telomerase RNA from the ciliate, *Glaucoma chatoni*, to the telomerase RNA of another ciliate, *Tetrahymena thermophila*, they observed shorter telomeres within the transfected (changed) cells. These two organisms have identical RNA template domains, but different non-template RNA sequences. When the nucleotides around the template domain changed, aberrant (abnormal) shorter telomeres resulted. Non-template RNA domains affect crucial molecular events taking place at the polymerization active site of telomerase. This finding suggests the possible control of telomerase activity by changing the molecular interactions involving its non-template RNA domains. (Bhattacharyya 1997)



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Austriaco, N R Jr. Guarente, l p 1997. "Changes of Telomere Length Cause Reciprocal Changes in the Lifespan of Mother Cells in *Saccharomyces Cerevisiae*." *Proc. Natl. Acad. Sci. USA*. Vol. 94:pp. 9768-9772.

Telomerase of *Saccharomyces cerevisiae* is inactivated by mutation and compared to a wild-type control group. Results contradict the telomere shortening theory of cellular senescence. The author believes telomeric length regulates gene silencing machinery available to non-telomeric DNA.

Autexier A, Pruzan R, Funk W D, and Greider C W. 1996 "Reconstitution of Human Telomerase Activity and Identification of a Minimal Functional Region of the Human Telomerase RNA." *EMBO J* Vol. 15:No.9 pp. 5928-5935

This article provides an in-depth description of the development of a functional *in vitro* reconstitution assay for the detection of telomerase activity.

Bednenko J, Melik M, Greene E C, and Shippen D E, 1997 "Developmentally Regulated Initiation of DNA Synthesis by Telomerase: Evidence for Factor-Assisted *de Novo* Telomere Formation." *EMBO J* Vol. 16:No.9 pp/ 2507-2518

These researchers look at programmed initiation of DNA synthesis in ciliates. Results indicate a dissociable chromosome healing factor works with telomerase to initiate programmed telomere formation.

Belair C D, Yeager T R, Lopez P M, Rexnikoff C A. 1997 "Telomerase Activity: A Biomarker of Cell Proliferation, NOT Malignant Transformation." *Proc. Natl. Acad. Sci. USA*. Vol. 94: pp 13677-13682

This article looks at the role of telomerase activity as a biomarker of proliferation in mortal and immortal human cell lines. Telomerase activity measurements reflected differences in proliferation between tumor and normal cell lines *in vivo*.

Bhattacharyya A and Blackburn E. 1997 "A Functional Telomerase RNA Swap *in Vivo* Reveals the Importance of Non-template RNA Domains." *Proc. Natl. Acad. Sci. USA*. Vol. 94: pp2823-2827

An interspecies telomerase RNA swap *in vivo* produces functional but aberrant telomerase. Results reflect a possible role of non-template telomerase RNA domains in regulating telomerase activity.

Bianchi A, Smith S, Chong L, Elias P, and Lange T. 1997 "TRF1 is a Dimer and bends Telomeric DNA." *EMBO J*. Vol. 16:No 7. pp1795-1805

Conformational interactions between DNA and associated proteins indicate DNA

bending plays an important role in telomere function in yeast and mammals. Bending of telomeric DNA by the TRF1 protein could account for the tight packaging of telomeres in interphase nuclei. Disruption of this packaging can effect telomere length.

Bodnar A G, Ouellette M, Frolkis M, Holt S E, Chiu C, Morin G B, Harley C B, Shay J W, Lichtsteiner S, and Wright W E 1998 "Extension of Life-Span by Introduction of Telomerase Into Normal Human Cells." *Science*. vol. 279:16 Jan. pp 349-352.

This research lends support to the telomere shortening theory of cellular senescence. Normal telomerase-negative human cells are transfected for telomerase activity. Induction of telomerase activity increases population doublings by at least twenty beyond the normal lifespan of the cell line.

Bugaeva E A, and Podgornaya O I. 1997 "Telomere-Binding Protein From the Nuclear Envelope of Oocytes of the Frog *Rana Temporaria*." *Biochemistry*. Vol. 62:n11 pp 1311-1318

The role of telomeres as structures that function to anchor chromosomes within the nucleus is examined. Telomeres are found to form a net-like structure attached to the nuclear envelope of frog oocytes.

Cech R R, Nakamura T M, and Lingner J. 1997 "Telomerase is a True Reverse transcriptase. A Review." *Biochemistry*. Vol. 62:n11 pp 1202-1207

An excellent review of the reverse transcriptase properties of the telomerase RNA template domain.

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Dionne I, and Wellinger R J. 1996 "Cell Cycle-Regulated Generation of Single Stranded G-Rich DNA in the Absence of Telomerase." *Proc. Natl. Acad. Sci. USA*. Vol. 93 pp13902-13907

This research raises questions regarding the importance of telomerase in telomere formation. A new in-gel hybridization technique reveals the addition of telomeric DNA to yeast chromosomes, during the S phase of mitosis, in the absence of telomerase.

Fan S, and Price C M. 1997 "Coordinate Regulation of G- and C-Strand Length During New Telomere Synthesis." *Molecular Biology of the Cell*. Vol. 8 pp. 2145-2155

Manipulation of the expression of various DNA related proteins provides an in-depth look at the tight regulation of telomere length. DNA polymerase is reported to regulate the coordination of G- and C-strand length of telomeric DNA.

Hale W G, and Margham J P. eds. 1991 *The Harper Collins Dictionary of Biology*. New York:HarperCollins Publishers.

Harrington L, McPhail T, Mar V, Zhou W, Oulton R, Amgen EST Program, Bass M B, Arruda I, Robinson M O. 1997 "A Mammalian Telomerase-Associated Protein." *Science*. Vol. 275 pp

973-977

The structure and function of the mammalian catalytic telomerase-associated protein, TP1, is compared to the *Tetrahymena* homolog, p80. The ciliate protein is shown to interact with mammalian telomerase RNA.

Harrington L, Zhou W, McPhail T, Oulton R, Young D S K, Mar V, Bass M B, and Robinson M O. 1997 "Human Telomerase contains Evolutionarily Conserved Catalytic and Structural Subunits." *Genes & Dev.* Vol. 11 pp 3109-3115

Mammalian telomerase-associated protein, TP2 is shown to associate with telomerase-associated protein, TP1. Authors discuss the possible homology with similar proteins in ciliates and yeast.

Hayflick L. 1997 "Mortality and Immortality at the Cellular Level. A Review." *Biochemistry.* Vol. 62:n11 pp1180-1189

An excellent review of the concepts of cellular mortality and immortality. Hayflick reports that there are no truly immortal cell lines. Various age related concepts are examined, including terminal differentiation versus aging.

Holt S E, Aisner D L, Shay J W, Wright W E. 1997 "Lack of Cell Cycle Regulation of Telomerase activity in Human cells." *Proc. Natl. Acad. Sci. USA* vol. 94 pp 10687-10692

These researchers try to resolve conflicts regarding telomerase activity and regulation during the various stages of the cell cycle. They found telomerase activity correlated with proliferative capacity and did not change significantly with progression through the cell cycle.

Ishikawa F. 1997 "Regulation Mechanisms of Mammalian Telomerase, A Review." *Biochemistry.* Vol. 62:n11 pp 1332-1339

A comprehensive review of the regulation of telomerase activity. This review includes a look at the genes encoding telomerase proteins, the structure of telomerase in various organisms, and telomerase activity during the cell cycle.

Lin J, and Zalian V A. 1996 "The *Saccharomyces* CDC13 Protein is a Single-Strand TG1-3 Telomeric DNA-Binding Protein *in Vitro* That Affects Telomere Behaviour *in Vivo*." *Proc. Natl. Acad. Sci. USA.* Vol. 93 pp 13760-13765

The role of Cdc13 in telomerase activity is examined. This yeast protein was found to bind to telomeric DNA protecting it from degradation and transcription while masking it from factors that detect damaged DNA *in vitro*. They also consider the genetic evidence for similar functions *in vivo*.

Lingner J, Cech T R, Hughes T R, and Lundblad V. 1997 "Three Ever Shorter Telomere (EST) Genes are Dispensable for *in Vitro* Yeast Telomerase Activity." *Proc. Natl. Acad. Sci. USA.* Vol. 94 pp 11190-11195

Four genes in yeast are believed to encode for telomerase proteins. These researchers

show that only one of these four, EST1, is essential for telomerase activity.

Linger J, Hughes T R, Shevchenko A, Mann M, Lundblad V, and Cech T R. 1997 "Reverse Transcriptase Motifs in the Catalytic Subunit of Telomerase." *Science*. Vol. 276 pp561-567

This research examines the importance of the fold in the ciliate telomerase associated protein, p123, and its function in telomere replication. A model portraying the telomerase molecule as a "hand" holding the telomerase RNA template and telomere, during replication, is presented.

McCormick-Graham M, Haynes W J, and Romero D P. 1997 "Variable Telomeric Repeat Synthesis in *Paramecium Tetraurelia* is Consistent with Misincorporation by Telomerase." *EMBO*. Vol. 16:n11 pp 3233-3242

Telomeres of most organisms consist of short tandem repeats of telomeric DNA. These researchers look at the causes of repeat variability in *Paramecium caudatum*. Telomerase RNA template substitutions is reported to be the likely cause of this repeat variability.

McElligott R, and Wellinger R J. 1997 "The Terminal DNA Structure of Mammalian Chromosomes." *EMBO J*. Vol. 16:no. 12 pp 3705-3714.

Human and mouse telomeres are examined using an indirect labeling method and primer extension protocol. The structure of mammalian telomeres is reported to consist of a G-rich overhang that is greater than or equal to 45 nucleotide bases long.

Mirabella A, and Gartenberg M R. 1997 "Yeast Telomeric Sequences Function as Chromosomal Anchorage Points *in Vivo*." *EMBO J*. Vol. 16:No. 3 pp 523-533

This research reports the ability of yeast telomeric DNA to block axial rotation of yeast DNA within the macronucleus. Anchoring capabilities of various telomerase/DNA related proteins are analyzed. Results support the formation of a macronuclear protein-DNA assembly linked to an insoluble portion of the nuclear membrane. The importance of DNA anchoring in telomere biology is discussed.

Nakamura T M, Morin G B, Chapman K B, Weinrich S L, Andrews W H, Lingner J, Harley C B and Cech T R. 1997 "Telomerase Catalytic Subunit Homologs From Fission Yeast and Human." *Science*. Vol. 277 pp 955-959

This work suggests that human telomere reverse transcriptases are closely related to RNA polymerases. The authors present a possible phylogenetic tree of telomerases and retroviruses stemming from RNA-dependent RNA polymerases.

Nugent C I, Hughes T R, Lue N F, and Lundblad V. 1996 "Cdc13p: A Single-Strand Telomeric DNA-Binding Protein with a Dual Role in Yeast Telomerase Maintenance." *Science*. Vol. 274 pp 249-252

This work examines the role of the yeast protein, Cdc13, in the regulation of telomerase. A model is presented that shows the protein protecting the chromosome end from degradation while mediating telomerase activity.

Prescott J, and Blackburn E. 1997 "Functionally Interacting Telomerase RNAs in the Yeast Telomerase Complex." *Genes & Dev.* Vol. 11:No. 21 pp2790-2800

This article details extensive research that shows telomerase remains stably bound to telomeric DNA in yeast and has two functional RNA template domains. A model is presented in which two distinct telomerase RNA template domains bind two different telomeric structures.

Rawes V, Kipling D, Kill I R, and Faragher R G A. 1997 "The Kinetics of Senescence in Retinal Pigmented Epithelial Cells: A Test for the telomere Hypothesis of Ageing." *Biochemistry.* Vol. 62:no. 11 pp1510-1515

This work challenges the telomere shortening hypothesis of aging. An examination of the kinetics of telomere shortening reveal that telomeres do not become shorter at the same rate within the same cell line.

Reveal P J, Henkels K M, and Truchi J J. 1997 "Synthesis of the Mammalian Telomere Lagging Strand in Vitro." *J. Of Bio. Che.* Vol. 272:No. 18 pp11678-11681

Results of this research confirm the RNA-dependent synthesis of the mammalian, lagging strand, telomeric DNA. Evidence for RNA priming and DNA extension as the mechanism for lagging strand replication is presented.

Russell P J, Ed. 1996 *Genetics.* p 375 R. R. Donelley & Sons Co.

Shen M, Haggbloom C, Bogt M, Hunter T, and Lu K P. 1997 "Characterization and Cell Cycle Regulation of the Related Human Telomeric Proteins Pin2 and TRF1 Suggest a Role in Mitosis." *Proc. Natl. Acad. Sci.* vo.. 94 pp 13618-13623

This research shows that the human telomeric repeat binding factor protein (TRF1) forms homo- and heterodimers with another human protein, Pin2. Results reveal that Pin2 binds directly to telomeres with a greater affinity than TRF1. These results indicate that Pin2 is the major human telomeric protein.

Vaziri H, West M D, Allsopp R C, Davison T S, We Y, Arrowsmith C H, Poirier G G, and Benchimol S. 1997 "ATM-Dependent Telomere Loss in Aging Human Diploid Fibroblasts and DNA Ploymerase." *EMBO J.* Vol. 16:no. 19 pp 6018-6033

Evidence is presented for a signaling function of shortened telomeres. Results indicate that shortened telomeres and damaged DNA activate the human protein, p53, which starts a process that causes cell cycle arrest in the G1 phase of the cell cycle.

Wang H, and Blackburn E H. 1997 "*De Novo* Telomere Addition by *Tetrahymena* Telomerase in Vitro." *EMBO J.* Vol 16:no. 4 pp866-879

These researchers examine the creation of telomeres at the end of non-telomeric DNA. In addition to the telomerase RNA template domain, they propose a second functional domain on the telomerase associated p95 protein. The lengths of telomeric and non-telomeric DNA strands, required for efficient telomere addition *in vitro*, are also reported.

Weng N, Granger L, and Hodes R J. 1997 "Telomere Lengthening and Telomere Activation During Human B Cell Differentiation." *Proc. Natl. Acad. Sci. USA*. Vol. 94 pp 10827-10832

This work examines telomere length and telomerase expression in the regulation of cellular lifespan of human B cells. Telomerase activity and telomere lengthening were found at higher rates in the germinal center. These results indicate the importance of telomerase in maintaining the replicative potential of immune B cell lymphocytes.

Yegorov Y E, Chernov D N, Akimov S S, Akhmalisheva A K, Smirnov Y B, Shinkarev D B, Semenova I V, Yegorova I N, and Zelenin A V. 1997 "Blockade of Telomerase Function by Nucleoside Analogs." *Biochemistry*. Vol. 62:No. 11 pp 1516-1526

Results of this research support the work of Bianchi, et al. These researchers show that shortening of telomeres within mouse embryonic cells does not produce cellular senescence. They propose a model for the signal that does induce cellular senescence, based on conformational changes in telomeric DNA.

Zhu S, Kumar R, Mandal M, Sharma N, Sharma H W, Dhingra U, Sololoski J A, Hsiao R, and Narayanan R. 1996 "Cell Cycle-dependent Modulation of Telomerase Activity in Tumor Cells." *Proc. Natl. Acad. Sci.* Vol. 93:No. 12 pp 6091-6095

Evidence for the cell cycle regulation of telomerase activity in tumor cells is presented in this report. Telomerase was observed to be highest in the S phase of the cell cycle and almost absent in the G2/M phase of the cycle. These results suggest a direct link between telomerase activity and progression through the cell cycle.

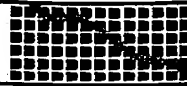


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Are you a mouse that panics?



Next Stop: Eternal Youth



Researchers took cells that were just about to die and rejuvenated them by cloning them. Using an enzyme that keeps a cell dividing, they hope to create an immortal 'cell line' of growing cells.

Cloned Calves

REUTERS

WASHINGTON, May 22 —

Researchers who have produced designer calves that are both cloned and genetically engineered said today their technique has given them a way to rejuvenate dying cells.

They said they hoped to eventually produce genetically engineered calves that carry a "fountain of youth" gene discovered by other teams of researchers.

Writing in the journal *Science*, Jose Cibelli and James Robl of the University of Massachusetts and colleagues described the technology they used to produce three Holstein calves born last January at a Texas ranch and three more born in March.

They did not clone the calves from adult animals, but from a fetus. But they said their technique held more commercial promise than that used to produce Dolly, the sheep who was the first mammal to be cloned from an adult cell.

Their work sheds light on which cells can be cloned and which cannot. It has to do with the cell's life cycle, and which stage it is in. But Robl said there was more.

"We also show that you can re-set the clock on these cells," he said.

Rejuvenated by Cloning

Robl said his team had taken cells that were just about to die, and rejuvenated them by cloning them. The process is known as nuclear transfer—taking the nucleus out of a cow's egg and then fusing another cell to the egg.

This "tricks" the egg into

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acting as if it had just been fertilized by a sperm. It starts developing into an embryo.

"Nuclear transfer is, essentially, the fountain of youth," Robl said.

The fetal cells that Robl's team used were fetal fibroblast cells—cells that have not yet differentiated into the tissues they will eventually become. Such cells still have the potential to make a whole animal rather than some specific tissue, such as connective tissue or an organ.

These cells usually divide about 30 times in a test tube before they simply stop—a condition known as senescence. What happens on the genetic level is that the telomeres, which cap and protect the ends of each chromosome, wear away.

"If you take cells that have gone to 29 cell divisions, and they are in the process of being ready just to stop and give up, and you use those for nuclear transfer, you rejuvenate them and they have the same lifespan as cells that came from the fetus originally," Robl said.

Eternal Cells

Robl has been speaking with Dr. Woodring Wright, a professor of cell biology at the University of Texas in Dallas, who reported in January on ways to make cells live essentially forever.

Wright's team used an enzyme known as telomerase, which is produced by egg cells and affects the telomeres. It probably helps the cells of a rapidly growing fetus keep dividing without wearing out. Normal adult cells do not produce telomerase.

Robl said he was now checking to see if the cells he uses to grow cattle clones have telomerase activity.

By injecting the gene that controls production of telomerase into the cells used to make clones, it may be possible to create an immortal "cell line" of

growing cells.

"Then you make a calf out of that cell line and see if you can make calves out of the adults," Robl said.

"I think it's a great experiment. I'm just anxious to get it done and I'm frustrated that we have to wait nine months to get an answer for it."

Wright has warned that simply re-activating telomerase would not be enough to make a whole animal live longer, that many processes are involved in aging.

But a calf containing an extra gene for telomerase would offer scientists a good way of studying the processes that underlie aging.

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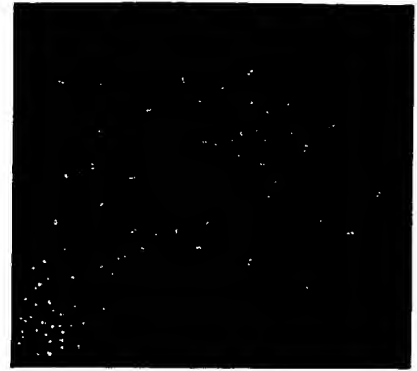
Jerry Shay, jshay@mednet.swmed.edu, University of Texas, Southwestern Medical Center, Dallas, USA

Definition: Telomerase (TE-LÓM-ER-ACE) is a ribonucleoprotein enzyme complex (a cellular reverse transcriptase) that maintains chromosome ends and has been referred to as a cellular immortalizing enzyme. Telomerase is a ribonucleoprotein reverse transcriptase enzyme (composed of both RNA and proteins) that uses its internal RNA component (complementary to the telomeric single stranded overhang) as a template in order to synthesize telomeric DNA (TTAGGG)_n, directly onto the ends of chromosomes. Telomerase is present in most fetal tissues, normal adult male germ cells, inflammatory cells, in proliferative cells of renewal tissues, and in most tumor cells. After adding six bases, the enzyme is thought to pause while it repositions (translocates) the template RNA for the synthesis of the next six base pair repeat. This extension of the 3' DNA template end in turn permits additional replication of the 5' end of the lagging strand, thus compensating for the end-replication problem.

Molecular Characteristics: Telomeres are the repetitive DNA sequences at the end of all linear chromosomes. In humans there are 46 chromosomes and thus 92 telomere ends that consist thousands of repeats of the six nucleotide sequence, TTAGGG. The telomere-telomerase hypothesis of aging and cancer is based on the findings that the cells of most human tumors have telomerase activity while normal human somatic cells do not. Telomere length is maintained by a balance between processes that lengthen telomeres (telomerase) and processes that shorten telomeres (the end replication problem). Telomerase is a cellular reverse transcriptase which stabilizes telomere length by adding hexameric TTAGGG repeats onto the telomeric ends of the chromosomes, thus compensating for the continued erosion of telomeres that occurs in its absence. The core catalytic subunit of telomerase, hTERT, is expressed in embryonic cells and in adult male germline cells, but is undetectable in normal somatic cells except for proliferative cells of renewal tissues (e.g. hematopoietic stem cells, activated lymphocytes, basal cells of the epidermis, proliferative endometrium, and intestinal crypt cells). The *hTERT* gene maps to chromosome band 5p15.33.

In normal somatic cells progressive telomere shortening is observed, eventually leading to greatly shortened telomeres and to a limited ability to continue to divide. Telomerase activity can be determined by the TRAP assay. It has been proposed that telomere shortening may be a molecular clock mechanism that counts the number of times a cell has divided and when telomeres are short cellular senescence (growth arrest) occurs. It has been proposed, but not proven, that shortened telomeres in mitotic (dividing) cells may be responsible for some of the changes we associate with normal aging. The length of telomeres can be visualized by demonstrating TRF (Terminal Restriction Fragments).

What are telomeres and what do they do? Telomeres are repeated DNA sequences that protect the ends of chromosomes from being treated like a broken piece of DNA needing repair. Without telomeres, the ends of the chromosomes would be "repaired", leading to chromosome fusion and massive genomic instability. Telomeres are also thought to be the "clock" that regulates how many times an individual cell can divide. Telomeric sequences shorten each time the DNA replicates. When at least some of the telomeres reach a critically short length, the cell stops dividing and ages (senesces) which may cause or contribute to some age-related diseases. In cancer, a special cellular reverse transcriptase, telomerase, is reactivated and maintains the length of telomeres, allowing tumor cells to continue to proliferate.

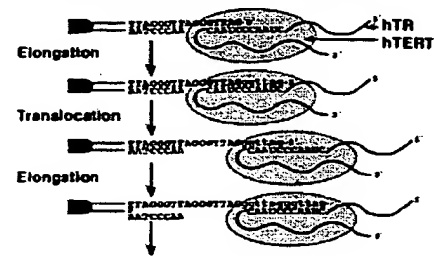


Telomeres in Human Chromosomes. Metaphase human chromosomes that have been both stained with DAPI (blue color which stains DNA/chromosomes) and also in situ hybridized with a PNA (peptide nucleic acid) fluorescently labelled telomere probe (red ends of each chromosome).

Why do telomeres shorten? The mechanisms of DNA replication in linear chromosomes is different for each of the two strands (called leading and lagging strands). The lagging strand is made as series of discrete fragments, each requiring a new RNA primer to initiate synthesis. The DNA between the last RNA priming event and the end of the chromosome cannot be replicated because there is no DNA beyond the end to which the next RNA primer can anneal, thus this gap cannot be filled in (this is referred to as the "end replication problem"). Since one strand cannot copy its end, telomere shortening occurs during progressive cell divisions. The shortened telomeres are inherited by daughter cells and the process repeats itself in subsequent divisions.

What is cellular senescence? In contrast to tumor cells, which can divide forever (are "immortal"), normal human cells have a limited capacity to proliferate (are "mortal"). In general, cells cultured from a fetus divide more times in culture than those from a child, which in turn divide more times than those from an adult. The length of the telomeres decreases both as a function of donor age and with the number of times a cell has divided in culture. There appear to be two mechanisms responsible for the proliferative failure of normal cells. The first, M1 (Mortality stage 1), occurs when there are still at least several thousand base pairs of telomeric sequences left at the end of most of the chromosomes. M1 may be induced by a DNA damage signal produced by one or a few of the 92 telomeres that have particularly short telomeres. The M1 mechanism causes a growth arrest mediated by the tumor suppressor genes *p16*, *RB1* and *p53*. If the actions of *p53* and *p16/pRB* are blocked, either by mutation or by binding to viral oncoproteins, then cells can continue to divide and telomeres continue to shorten until the M2 (Mortality stage 2) mechanism is induced. M2 represents the physiological result of critically short telomeres when cells are no longer able to protect the ends of the chromosomes, so that end-degradation and end-to-end fusion occurs and causes genomic instability and cell death. In cultured cells, a focus of immortal cells occasionally arises. In most cases, these cells have reactivated the expression of telomerase, which is able to repair and maintain the telomeres.

Telomerase: A Reverse Transcriptase Extends and Maintains Telomeres



Telomerase: A Cellular Reverse Transcriptase Extends and Maintains Telomeres. Telomeric sequences are synthesized by telomerase, a ribonucleoprotein enzyme (composed of both RNA and protein). Telomerase contains RNA-dependent DNA polymerase activity which uses its RNA component (complementary to the telomeric single stranded overhang) as a template in order to synthesize TTAGGG repeats (elongate) directly onto telomeric ends. After adding six bases, the enzyme is thought to pause while it repositions (translocates) the template RNA for the synthesis of the next six bp repeat. This extension of the 3' DNA template end in turn permits additional replication of the 5' end of the lagging strand, thus compensating for the telomere shortening that occurs in its absence.

If you can stop the shortening of telomeres will this prevent cellular aging? While there have been many studies indicating that there is a correlation between telomere shortening and proliferative failure of human cells, the evidence that it is causal has only recently been demonstrated. Introduction of the telomerase catalytic protein component into normal human cells without detectable telomerase results in restoration of telomerase activity. Normal human cells stably expressing transfected telomerase demonstrate extension of lifespan, providing more direct evidence that telomere shortening controls cellular aging. The cells with introduced telomerase maintain a normal chromosome complement and continue to grow in a normal manner. Initial concerns that the introduction of telomerase into normal cells may substantially increase the risk of cancer have not proven true. One way to think about this is that special reproductive tissues maintain high levels of telomerase throughout life, and there is no increased incidence of cancers in these special cells when compared with other types of cancer. Thus, the major role of telomerase is to maintain telomere stability and keep the cells dividing. These observations provide the first direct evidence for the hypothesis that telomere length determines the proliferative capacity of human cells.

Can telomerase be used as a product to extend cell lifespan? The ability to immortalize human cells and retain normal behavior holds promise in several areas of biopharmaceutical research including drug development, screening and toxicology testing. The development of better cellular models of human disease and production of human products are among the immediate applications of this new advance. This technology has the potential to produce unlimited quantities of normal human cells of virtually any tissue type and may have most immediate translational applications in the area of transplantation medicine. In the future it may be possible to take a person's own cells, manipulate and rejuvenate them without using up their lifespan and then give them back to the patient. In addition, genetic engineering of telomerase-immortalized cells could lead to the development of cell-based therapies for certain genetic disorders such as muscular dystrophy.

Cell and molecular regulation. There are proteins identified that directly interact with telomerase such as p23/hsp90 (molecular chaperones) and TEP1 (telomerase associated protein 1 with unknown function). In addition, there are likely to be other proteins that help regulate telomerase function that have yet to be identified. The transcriptional regulation of the catalytic subunit of telomerase (hTERT) is clearly complex, but there is recent evidence that the gene may be important in some aspects of the transcriptional activation of hTERT. In addition, there is evidence that a gene on chromosome 3 may be involved in the transcriptional repression of hTERT. Since

telomerase interacts with the telomeres, there has been a number of proteins identified that directly or indirectly bind to telomeres (TRF1, TRF2, tankyrase, TIN2) that are also important in the regulation of telomerase. There is also regulation of the level of telomerase activity in specific cell types. In the most primitive stem cells of renewal tissues (e.g. crypts of the intestine, bone marrow cells, resting lymphocytes, basal layer of the epidermis) telomerase activity is low while in the proliferative descendants of these cells telomerase activity is increased. Thus, there are telomerase competent cells that have low activity when quiescent (not dividing) and increased activity when proliferating (dividing). However, these telomerase competent stem cells do not fully maintain telomere length since such cells obtained from older individuals have shorter telomeres than those derived from younger individuals. Thus, in germline (reproductive) cells and tumor cells, telomerase fully maintains telomere length in contrast to stem cells (with regulated telomerase activity) and most somatic cells (with no detectable telomerase activity) in which telomeres progressively shorten with increased age.

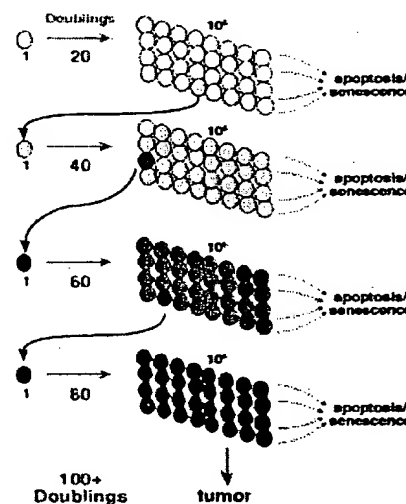
Biological Activity in Cancer: Cellular senescence may have evolved, in part, to protect long-lived organisms, such as humans, against the early development of cancer. Thus, it has been proposed that upregulation or reexpression of telomerase may be a critical event responsible for continuous tumor cell growth. In contrast to normal cells, tumor cells show no net loss of average telomere length with cell division, suggesting that telomere stability may be required for cells to escape from replicative senescence and proliferate indefinitely. Most, but not necessarily all, malignant tumors may need telomerase to sustain their growth. Immortalization of cells may occur through a mutation of a gene in the telomerase repression pathway. Thus, upregulation or reactivation of telomerase activity may be a rate-limiting step required for the continuing proliferation of advanced cancers. There is experimental evidence from hundreds of independent laboratories that telomerase activity is present in approximately 90 percent of all human tumors but not in tissues adjacent to the tumors. Thus, clinical telomerase research is currently focused on the development of methods for the accurate diagnosis of cancer and on novel anti-telomerase cancer therapeutics.

Clinical relevance: There is mounting evidence that cellular senescence acts as a "cancer brake" because it takes many divisions to accumulate all the changes needed to become a cancer cell. In addition to the accumulation of several mutations in oncogenes and tumor suppressor genes, almost all cancer cells are immortal and, thus, have overcome the normal cellular signals that prevent continued division. Young normal cells can divide many times, but these cells are not cancer cells since they have not accumulated all the other changes needed to make a cell malignant. In most instances a cell becomes senescent before it can become a cancer cell. Therefore, aging and cancer are two ends of the same spectrum. The key issue is to find out how to make our cancer cells mortal and our healthy cells immortal, or at least longer-lasting. Inhibition of telomerase in cancer cells may be a viable target for anti-cancer therapeutics while expression of telomerase in normal cells may have important biopharmaceutical and medical applications. In summary, telomerase is both an important target for cancer and for the treatment of age-related disease.

Could telomerase be the "Achilles heel" of cancer? We believe that progressive telomere shortening is halted in cancer cells by the presence of the enzyme telomerase which maintains and stabilizes the telomeres, allowing cells to divide indefinitely. Telomerase activity is detected in almost all human tumors. It is hoped that a therapy can be developed that inhibits telomerase activity and interferes with the growth of many types of cancer.

Will inhibiting telomerase restore the senescence program in cancer cells and if so will this therapy cure cancer? One research strategy is to inhibit the activity of telomerase, forcing immortal cells into a normal pattern of permanent growth arrest (senescence) or death (apoptosis). Following conventional treatments (surgery, radiotherapy, chemotherapy) anti-telomerase agents would be given to limit the proliferative capacity of the rare surviving tumor cells in the hope that this would prevent cancer recurrence. We believe this treatment would be very selective in that only cells with an activated telomerase would be affected. As far as we know, that includes only "immortal" tumor cells and germline (reproductive cells) and at lower levels stem cells in renewal tissues.

Will telomerase activity be useful in cancer diagnostics? Telomerase activity is detected in premalignant specimens (*in situ* lung and breast



Telomerase: A Cellular Reverse Transcriptase Extends and Maintains Telomeres. It has been argued that it may not be necessary to exhaust the replicative potential of normal cells in order to form a massive tumor (e.g. after 50 doublings a single cell could generate a tumor of a size greater than 1000 kilograms). However, this theoretical argument assumes that all cells survive, which is highly unlikely to be correct. If the frequency of spontaneous mutations is approximately 10^{-6} , at least a million cells are needed for a second mutation to occur with reasonable probability. Since these mutations must accumulate in the same cell, a series of clonal expansions must occur as is illustrated in this figure. Since it requires 20 cell doublings to generate approximately one million cells, 20 divisions would accompany each mutation. For example if we assume five mutations are necessary for cancer to arise from a normal cell, more than 100 divisions

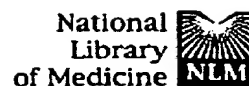
cancers), while colon and pancreatic cancer have detectable telomerase activity at later (carcinoma) stages. The ability to use almost any clinical specimen and to demonstrate telomerase may allow the detection of cancers at an earlier stage. Telomerase activity is detected in lung cells in cancer patients obtained by bronchial alveolar lavage. In addition, fine needle aspirations (breast, liver and prostate cancer), washes (bladder and colon), and sedimented cells from urine (bladder and prostate) provide minimally invasive sources of cells to detect telomerase activity and are likely to have immediate diagnostic utility as well as monitoring of minimal residual disease. In an effort to improve the diagnostic value of telomerase determinations, *in situ* hybridization methods for the demonstration of telomerase on archival paraffin embedded clinical specimens appears to distinguish cancer from normal cells, correlates well with telomerase activity, and thus may provide added value to telomerase activity assays. In addition, the presence or absence of telomerase may have prognostic value and help risk stratify patients into those with favorable outcomes (to avoid unnecessary treatments for patients with low or no detectable telomerase) and those with high telomerase activity and with unfavourable outcomes (to help oncologists manage patient treatments more effectively).

from a normal cell, more than 100 divisions (doublings) would be required to render a cell malignant. Losses of cells due to apoptosis or inhibition of cell proliferation due to senescence would limit the number of cells in a such a tumor to 10^6 - 10^8 cells which is less than a 1 gram biomass. Thus, with the possible exception of certain stem cells from the bone marrow, skin, and perhaps the intestine, most normal human cells only divide 50-70 times before they growth arrest. Thus cellular senescence could act as a very effective »brake« on the proliferation of cells that had accumulated a few mutations but not all those prerequisite for malignancy.

Have any telomerase theapeutic agents been identified and what are the potential complications of such strategies? Since telomerase is expressed in most advanced cancers, methods for telomerase inhibition using small molecules such as modified oligonucleotides may have utility. There are potential risks in the use of such therapy that must be considered, for example the affects of inhibitors on telomerase-expressing stem cells. However, it is likely that this approach will be less toxic than conventional chemotherapy which affects all proliferating cells, including stem cells. The rate of division of the most primitive stem cells is so much slower than that of most cancer cells that the amount of telomere shortening in the stem cells should be relatively small. Some of the side effects of standard chemotherapy, such as thrombocytopenia, leukopenia, nausea and hair loss due to the death of the cells in rapidly proliferating tissues, may be reduced by the use of telomerase inhibitors which are predicted to induce cellular senescence only after prolonged growth. This raises what many consider the most important concern with this proposed treatment regimen, the prolonged time potentially required for a telomerase inhibitor to be effective. Since the mode of action of telomerase inhibitors may require telomeric shortening before inhibition of cell growth or induction of apoptosis, there may be a significant delay in efficacy. Thus methods may have to be devised to increase the rate of telomere shortening when telomerase inhibitors are used therapeutically. Telomerase inhibitors will likely be used together with or following conventional therapies, so that once the bulk of the tumor mass is eliminated anti-telomerase therapy might prevent the large number of cell divisions required for the regrowth of rare resistant cancer cells. They may also be used in early stage cancer to prevent overgrowth of metastatic cells, as well as in high-risk patients with inherited-susceptibility to cancer syndromes to prevent the emergence of telomerase-expressing cells (chemoprevention).

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Repression of the telomerase catalytic subunit by a gene on human chromosome 3 that induces cellular senescence.

Horikawa I, Oshimura M, Barrett JC.

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The cellular senescence program is controlled by multiple genetic pathways, one of which involves the regulation of telomerase and telomere shortening. The introduction of a normal human chromosome 3 into the human renal cell carcinoma cell line RCC23 caused repression of telomerase activity, progressive shortening of telomeres, and restoration of the cellular senescence program. We attributed the repression of telomerase activity to the marked downregulation of the gene encoding the catalytic subunit of telomerase (hEST2/hTERT) but not another protein component (TP1/TLP1) or the RNA component of telomerase. These results suggest that a senescence-inducing gene on chromosome 3 controls hEST2/hTERT gene expression either directly or indirectly and support the notion that hEST2/hTERT is the major determinant of telomerase enzymatic activity in human cells.

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